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# Unmyelinated trigeminal pathways as assessed by laser stimuli in humans

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## Summary

**Laser pulses excite superficial free nerve endings innervated by small-myelinated (Aδ) and unmyelinated (C) fibres. Whereas laser-evoked scalp potentials (LEPs) are now reliably used to assess function of the Aδ-fibre nociceptive pathways in patients with peripheral or central lesions, the selective activation of C-fibre receptors and recording of the related brain potentials remain difficult. To investigate trigeminal C-fibre function, we directed laser pulses to the facial skin and studied sensory perception and scalp evoked potentials related to Aδ- or C-fibre activation in healthy humans and patients—one having a bilateral facial palsy, two a trigeminal neuropathy, and two a Wallenberg syndrome. We also measured afferent conduction velocity and, with source analysis, studied the brain generators. Whereas laser pulses of low intensity and small irradiated area elicited pinprick sensations and standard Aδ-LEPs, laser pulses of very-low intensity and large irradiated area elicited warmth sensations and scalp potentials with a latency compatible with C-fibre conduction (negative wave 280 ms, positive wave 380 ms); the estimated conduction velocity was 1.2 m/s. The main waves of the scalp potentials originated from the anterior cingulate gyrus; they were preceded by activity in the opercular region and followed by activity in the insular region. The patient with bilateral facial palsy, who had absent trigeminal-facial reflexes, had normal Aδ- and C-related scalp potentials; the patients with trigeminal neuropathy, characterized by loss of myelinated and sparing of unmyelinated fibres, had absent Aδ- but normal C-related potentials; and the patients with Wallenberg syndrome had absent Aδ- and C-related potentials. We conclude that laser pulses with appropriate characteristics evoke brain potentials related to the selective activation of trigeminal nociceptive Aδ or thermal C fibres. The trigeminal territory yields rewarding LEP findings owing to the high density of thermal receptors and, because the short conduction distance, minimizes the problem of signal dispersion along slow-conducting unmyelinated afferents. The opercular-insular region and the cingulate gyrus are involved in the processing of C-fibre trigeminal inputs. The method we describe may prove useful in patients with lesions affecting the trigeminal thermal pain pathways.**

**Keywords**: trigeminal nerve; unmyelinated fibres; laser evoked potentials; thermal-pain perception; trigeminal nerve biopsy

**Abbreviations**: Aδ-LEP and C-LEP = laser evoked potentials after selective activation of small-myelinated (Aδ) and unmyelinated (C) afferents; LEPs = laser-evoked scalp potentials; Nd:YAP = neodymium-doped yttrium aluminium perovskite; SI and SII = primary and secondary somatosensory cortex

## Introduction

Radiant heat pulses delivered by high-power laser stimulators and directed to the hairy skin excite superficial free nerve endings innervated by small-myelinated (Aδ) and unmyelinated (C) fibres. Laser-evoked scalp potentials (LEPs) are now reliably used to assess function of the Aδ-fibre nociceptive pathways in patients with peripheral neuropathy or central lesions (Bromm and Treede, 1991; Kakigi *et al*., 1991, 1992; Treede *et al*., 1991), including mandibular neuropathy, trigeminal neuralgia, and Wallenberg syndrome, which impair the trigeminal system (Cruccu *et al*., 1999, 2001). Although the selective activation of C-fibre receptors and recording of the related brain potentials are more

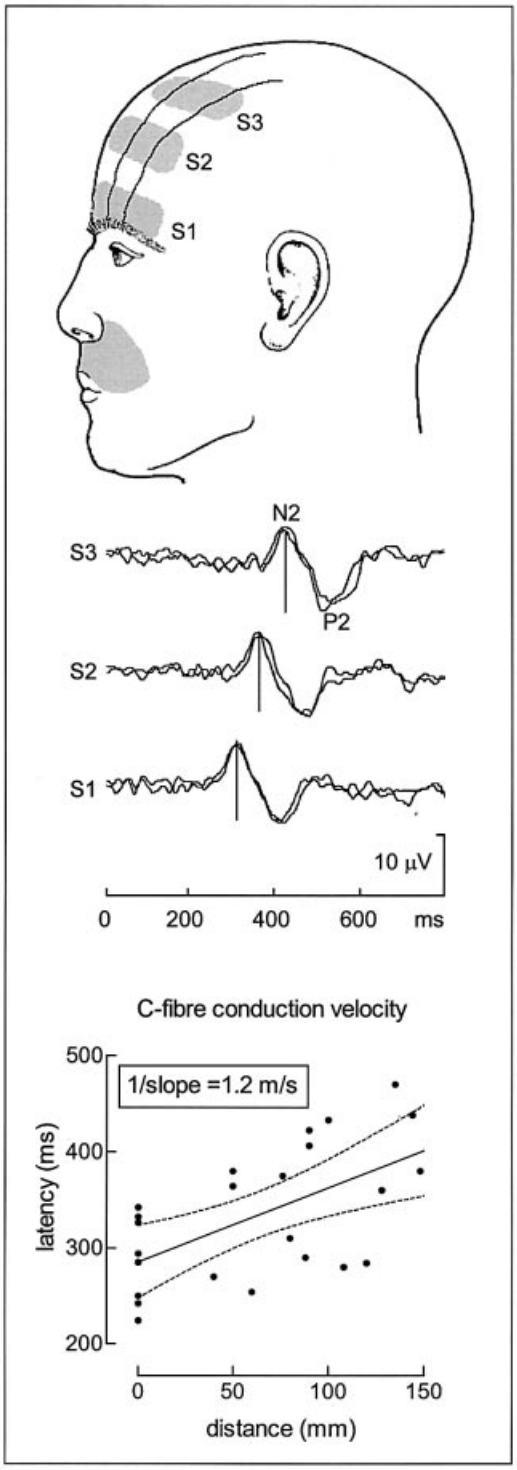


**Fig. 1** Conduction velocity of unmyelinated afferents. *Upper panel*: schematic drawing of the three areas of stimulation (S1, S2, and S3) along the course of the supraorbital nerve branches (the grey area below the nose depicts the territory of highest sensitivity). *Middle panel*: C-fibre related LEPs after stimulation (24 mJ/mm$^2$, 177 mm$^2$) of the forehead skin at S1, S2, and S3 in a representative subject. Two superimposed averages of 20 trials each. *Lower panel*: scatterplot of the LEP latencies (taken at the N2 peak) at all stimulation sites in all subjects. Regression line ($P < 0.005$) indicated by continuous line, 95% confidence intervals by dashed lines.

difficult, C-fibre evoked potentials after hand and foot stimulation have been described in healthy humans (Bromm *et al*., 1983; Bragard *et al*., 1996; Towell *et al*., 1996; Magerl *et al*., 1999) and patients (Treede *et al*., 1988, 1991; Lankers *et al*., 1991; Granot *et al*., 2001). These studies used various techniques: experimental block of group A fibres (Bromm *et al*., 1983); spectral analysis of the expected time window (Arendt-Nielsen, 1990; Bragard *et al*., 1996); selection of single trials devoid of Aδ-LEPs (Towell *et al*., 1996); 'microspot' stimulation (Bragard *et al*., 1996; Opsommer *et al*., 1999); or stimulus intensities below the Aδ activation threshold (Treede *et al*., 1995; Magerl *et al*., 1999; Iannetti *et al*., 2003).

To select further between C nociceptors and C warmth receptors, the investigators exploited differences in their threshold and density. C warmth receptors have a slightly lower threshold than C nociceptors and a far lower density in the skin (LaMotte and Campbell, 1978; Tillman *et al*., 1995; Green and Cruz, 1998). Hence, the highest probabilities of selective activation for warmth receptors are yielded by low-intensity laser pulses irradiating a large skin area (Towell *et al*., 1996; Agostino *et al*., 2000; Iannetti *et al*., 2003) and those for C nociceptors by stimulation of very small areas (~0.2 mm) (Bragard *et al*., 1996; Opsommer *et al*., 1999; Qiu *et al*., 2001; Tran *et al*., 2002).

To assess trigeminal C-fibre function, we used laser pulses directed to the facial skin and recorded the related brain potentials in healthy humans. To verify that these brain potentials were generated by the thermal-pain sensory system, we also studied five patients: one with bilateral facial palsy; two with trigeminal neuropathy; and two with Wallenberg syndrome.

## Subjects and methods

Sixteen healthy volunteers (authors, research staff, PhD students and residents of the School of Neurology, La Sapienza University, Rome), aged 22–52 years, and five patients participated in the study. Patient 1 was a 27-year-old man, who had a head injury 2 weeks before examination; bilateral fractures at the base of the petrous bone had caused a bilateral facial palsy. Patients 2 and 3 were 63- and 64-year-old men, both with trigeminal neuropathy; Patient 2 had undergone a supraorbital nerve biopsy. Patient 4 was a 61-year-old man and Patient 5 a 44-year-old woman; both had a Wallenberg syndrome. MRI scans showed an ischaemic infarction in the dorsal-lateral medulla, on the left side in Patient 4 and the right in Patient 5. In these patients, we also assessed trigeminal function by recording the blink reflex, masseter inhibitory reflex, jaw jerk and motor evoked potentials after transcranial magnetic stimulation (Cruccu *et al*., 1989; Ongerboer de Visser and Cruccu, 1993).

All subjects and patients gave informed consent according to the Declaration of Helsinki and the Comitato Etico Ricerche Neurofisiologiche Dipartimento Scienze Neurologiche approved the procedures.

## *Laser stimulation*

We used two kinds of stimulator: a $CO_2$-laser (wavelength 10.6 μm, pulse duration 5–50 ms, maximum energy 1.5 J) commonly used in clinical practice and a neodymium: yttrium-aluminium-perovskite laser (Nd:YAP) (wavelength 1.34 μm, duration 1–10 ms, maximum energy 7 J) with fibre-optic guidance. Both were produced by Electronic Engineering, Florence, Italy. In preliminary experiments, we found that $CO_2$-laser pulses (pulse duration 30–50 ms, irradiated area ~20 $mm^2$, intensity 6–11 $mJ/mm^2$), elicited warmth sensations and brain potentials (C warmth input) if directed to the region of the upper-lip, in particular close to the sulcus nasus-genius and ala nasi. Stimuli directed elsewhere failed to evoke a purely warmth sensation or brain potentials. We then tried other laser stimulators and found that the Nd:YAP laser proved optimal to elicit warmth sensations in all facial territories. Nd:YAP laser pulses also had the advantage, as reported for the thulium laser (Spiegel *et al*., 2000), of inducing no damage to the facial skin—not even the transient dyschromic spots sometimes produced by $CO_2$-lasers (Cruccu *et al*., 1999, 2001; Romaniello *et al*., 2002).

Nd:YAP laser pulses of low intensity (16–37 $mJ/mm^2$), relatively-long duration (10 ms) and large irradiated area (~180 $mm^2$), raising the temperature of the facial skin to 39°C [as measured with a thermocouple (Iannetti *et al*., 2003)], were optimal to elicit purely warmth sensations (C-input). Pulses of higher intensity (49–76 $mJ/mm^2$), short duration (1 ms), and small irradiated area (~5 $mm^2$), raising the temperature to 48°C, were optimal to elicit pinprick sensations (Aδ-input).

Laser pulses were directed to the perioral or supraorbital skin. The irradiated spot was slightly shifted after each stimulus to avoid receptor sensitisation, and stimuli were delivered arrhythmically with 10–30 s intervals to minimize central habituation. Sensory thresholds were determined with series of increasing and decreasing stimulus-intensities (Cruccu *et al*., 1999; Agostino *et al*., 2000). Subjects were asked to describe the evoked sensation by choosing one of the following descriptors: 'touch'; 'pinprick'; 'warmth'; 'burning'; or 'it's difficult to describe'.

## *Scalp recordings*

Participants were seated in a dentist's chair and wore protective goggles. White noise was given through earphones. Subjects were instructed to keep their eyes open and gaze slightly downwards. In all subjects, the signals were recorded with disk electrodes from the vertex and referenced to linked earlobes (bandwidth 0.3–30 Hz). Simultaneous electroculography monitored ocular movements or eye-blinks. Two series of 20 artifact-free trials were collected and averaged off-line. We measured the peak latencies of the main negative (N2 wave) and positive (P2 wave) components and their peak-to-peak amplitude (Fig. 1).

To estimate the afferent conduction, we stimulated the supraorbital skin at three sites along the course of the supraorbital nerve branches in eight subjects (Fig. 1). We drew three points on the forehead skin: the most proximal above the eyebrow; the most distal on the scalp; and the third midway between the two. For each point, stimuli were delivered to an area of ~4 × 2 cm. To ensure a reproducible input and thus a similar level of spatial summation at central synapses, we set the stimulus intensity at the same multiples of the perceptive threshold at each area of stimulation. To avoid receptor sensitization or fatigue or a different level of habituation, we alternated the proximal, intermediate and distal areas of stimulation. Finally, we measured the LEP latencies (at the peak of the N2 wave) and distances, and calculated the slope of the total regression line obtained from the 24 couples of samples (Cruccu *et al*., 2000; Iannetti *et al*., 2003).

## *Electrical source analysis*

Ten subjects also underwent multi-electrode recordings (31-channel cap plus 1 EOG channel) to calculate a hypothesis-based model of a dipolar generator of the scalp signal. Using a Quick Brain System 98 (Micromed, Treviso, Italy), we averaged three blocks of 20 artifact-free trials after stimulation of the right perioral region for each subject and examined the grand-average (60 × 10). We performed dipolar source modelling using Brain Electrical Source Analysis (BESA) with a 'sequential strategy' as described in detail elsewhere (Valeriani *et al*., 2001). The model was built initially from the grand-average traces calculated across all 10 subjects; it was then applied to the individual C-related LEPs. BESA is a program that uses the surface-recorded EEG to estimate the source activities generating the scalp EP topography and then verifies whether the hypothesized dipolar model accounts for the recorded traces. The percentage of the recorded signal that cannot be explained by the dipolar model is indicated as residual variance (RV) (Scherg, 1990; Bromm and Chen, 1995). The dipole locations were expressed by Talairach's coordinates and converted into the Montreal Neurological Institute's MRI template (Evans *et al*., 1993).

## *Statistics*

We analysed intra-individual differences using the Wilcoxon matched-pairs test. Because the variance of latency values differed significantly between some groups, we assessed mean differences between groups with Welch's corrected test. We assessed the significance of the regression line used for estimating conduction velocity with the $r$ correlation index of linear regression and the deviation from zero of the slope with $F$ test. For all statistics and graphs, we used Prism 3.0 (GraphPad, Sorrento Valley, CA, USA). Throughout the text and tables, data are given as means ± 1SD.

**Table 1** *Latency and amplitude of trigeminal LEPs (mean ± SD)*

| Input and laser | Perioral | | | | | | Supraorbital | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N2 wave latency | | P2 wave latency | | Amplitude | | N2 wave latency | | P2 wave latency | | Amplitude | |
| | *n* | (ms) | *n* | (ms) | *n* | (μV) | *n* | (ms) | *n* | (ms) | *n* | (μV) |
| Aδ-fibre Nd:YAP | 16 | 162 ± 15 | 16 | 242 ± 25 | 16 | 21 ± 8 | 16 | 166 ± 35 | 16 | 246 ± 25 | 16 | 18 ± 11 |
| C-fibre Nd:YAP | 14 | 276 ± 32 | 16 | 377 ± 48 | 16 | 16 ± 5 | 10 | 289 ± 39 | 10 | 375 ± 57 | 10 | 15 ± 5 |
| [1]C-fibre $CO_2$ | 10 | 297 ± 30 | 10 | 404 ± 38 | 10 | 13 ± 5 | | – | | – | | – |
| [2]P | | NS | | NS | | NS | | | | | | |

[1]Stimulation confined within the region between upper lip and sulcus nasus-genius; [2]Statistical significance of differences between C-fibre LEPs after Nd:YAP and $CO_2$ stimulations (Welch's test). NS = not significant.

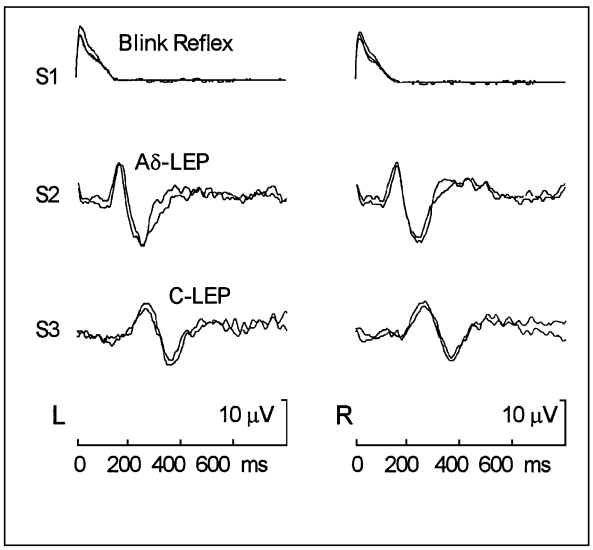


**Fig. 2** Findings in a patient with bilateral facial palsy (Patient 1). Right- and left-side responses. Supramaximal electrical stimulation failed to evoke reflex responses (S1). Laser pulses set for Aδ-fibre activation (S2, 50 mJ/mm$^2$, 4.9 mm$^2$) or C-fibre activation (S3, 20 mJ/mm$^2$, 177 mm$^2$), evoked the corresponding late and ultralate evoked potentials (Aδ-LEP and C-LEP). Stimulation of left (L) and right (R) sides. Note that calibration changes in S1: sweep 100 ms; sensitivity 50 μV.

## Results

### *Sensory perception*

When determining the sensory threshold with laser pulses optimal for Aδ-fibre activation, one subject reported a 'slight feeling of warmth', two said that they could not describe the sensation, and 13 reported a 'slight pinprick'. At the intensity used for evoked potential recording (1.5–2 × sensory threshold), all subjects reported a clear 'pinprick' sensation.

When the sensory threshold was determined with laser pulses optimal for C-fibre activation, all subjects reported a 'slight warmth'. At the intensity used for evoked potential recording (1.5 × sensory threshold), all subjects reported a clear 'warmth' sensation; no-one reported 'burning'.

### *LEPs*

In all subjects, stimuli set for Aδ-fibre activation evoked vertex potentials consisting of a negative wave at 160 ms and a positive wave at 240 ms, similar to those commonly elicited by $CO_2$ lasers (Cruccu *et al.*, 1999, 2001). Perioral and supraorbital stimuli gave similar results (Table 1).

In all subjects, stimuli set for C-fibre activation and directed to the perioral region elicited clear scalp potentials, consisting of a widespread negative–positive complex (N2 wave 280 ms; P2 wave 380 ms), with maximum amplitude at the vertex. In two subjects, however, the N2 wave was small and poorly reproducible (Table 1). In 10 subjects, we delivered stimuli set for C-fibre activation to the supraorbital skin. Again all subjects had a clear and reproducible P2 wave with maximum amplitude at the vertex and, in one subject, the N2 wave was poorly reproducible**.** LEPs after perioral and supraorbital stimulations did not differ significantly (Wilcoxon, $P > 0.10$). The intra-individual latency difference between C-related and Aδ-related LEPs always exceeded 100 ms (Wilcoxon, $P < 0.0001$).

After $CO_2$ laser stimulation of the upper lip, LEPs had a slightly longer latency and lower amplitude than those after Nd:YAP stimulations; the differences were not significant (Table 1).

### *Conduction velocity*

Probably because the available conduction distance was relatively short (maximum 150 mm) and the Aδ afferents have a comparatively high conduction velocity, no significant correlation was found between their latency and distance.

In contrast, the low conduction velocity of unmyelinated afferents yielded large differences in the latency of C-related LEPs elicited along the course of the supraorbital nerve. Stimulation at proximal and distal sites yielded LEPs of similar amplitude (Welch's test, $P > 0.10$). The regression line between latency and distance for all the 24 stimulated sites was highly significant ($r = 0.6269$, $P < 0.005$), as was the deviation of the slope from zero ($F = 10.36$, $P < 0.005$). The conduction velocity, indicated by the reciprocal of the slope, was 1.2 m/s (Fig. 1). Individual velocities ranged from 0.7 to 1.6 m/s.

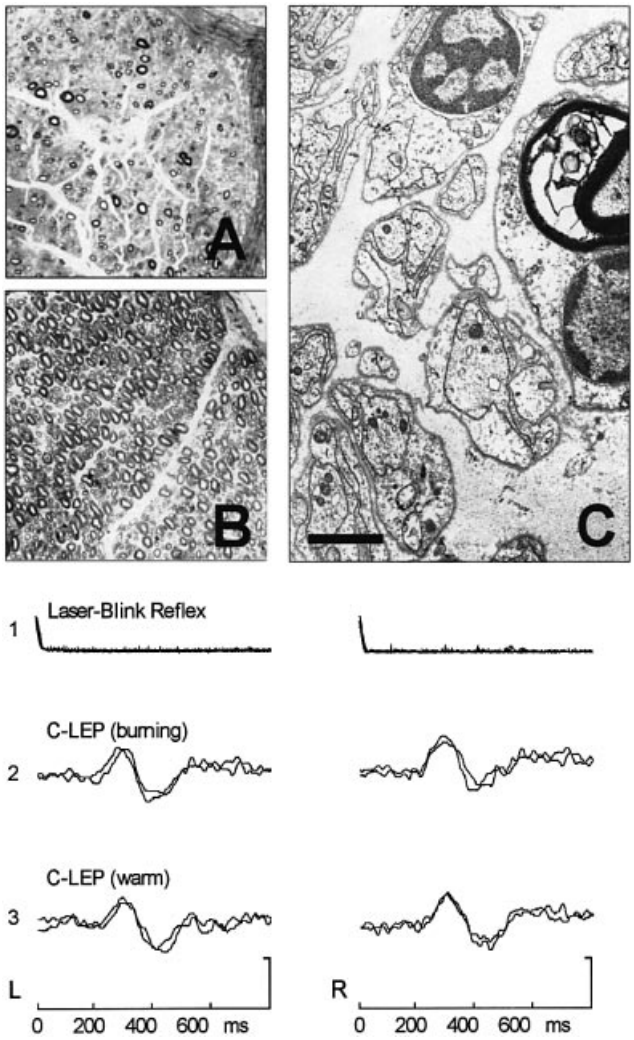


**Fig. 3** Histological and neurophysiological findings in a patient with trigeminal neuropathy (Patient 2). Optic microscopy of a supraorbital nerve fascicle shows severe loss of myelinated fibres (**A**) compared with a normal supraorbital nerve (**B**) [magnification ×25 for (**A**) and (**B**)]. (**C**) Electron microscopy (magnification bar 1 μm) shows sparing of unmyelinated fibres and one Aδ fibre in simil-Wallerian degeneration. *Lower panel*: responses to laser stimulation of the perioral region. High-intensity laser pulses set for Aδ-fibre activation (100 mJ/mm$^2$, 4.9 mm$^2$) elicited burning instead of pinprick sensations, failed to elicit the Aδ-mediated laser-evoked blink reflex in the orbicularis oculi muscle (1), and elicited C-like scalp potentials (2). Laser pulses set for C-fibre activation (30 mJ/mm$^2$, 113 mm$^2$) elicited warm sensations and normal C-LEPs (3). Stimulation of left (L) and right (R) sides. Sensitivity: 50 μV in 1 and 10 μV in 2 and 3.

## *Patients*

In Patient 1, who had a bilateral facial palsy, EMG examination showed complete absence of voluntary activity and absence of all trigeminal facial reflexes bilaterally. But he had normal pinprick and warmth thresholds, as well as normal Aδ-related and C-related LEPs (Fig. 2).

In Patient 2, who had trigeminal neuropathy, a sensory deficit in the intra- and perioral region had begun at 46 years and had progressed slowly to involve all the face bilaterally. At the age of 57 years, all the trigeminal reflexes including the blink reflex, the masseter inhibitory reflex and the jaw jerk were absent, whereas trigeminal motor function was normal; a supraorbital-nerve biopsy, compared with those of other patients (Pennisi *et al*., 1997), showed severe degeneration of myelinated axons, whereas unmyelinated fibres had a normal density (35 000/mm$^2$) and showed no collagen pockets or other histological abnormality on electron microscopy (Fig. 3). When we performed the laser study, the patient was 63 years old; he had probably lost further myelinated fibres because he had completely lost sharp–dull discrimination in all trigeminal territories. The laser-evoked blink reflex, a response mediated by Aδ-afferents (Romaniello *et al*., 2002) was absent bilaterally. Laser pulses set for eliciting Aδ-related LEPs, even at high stimulus intensities, failed to elicit pinprick sensations; the patient felt burning sensations and the scalp signals had a latency compatible with unmyelinated afferent activation. Laser pulses irradiating a large spot revealed a normal warmth threshold and elicited normal C-related LEPs (Fig. 3). Patient 3 reported pain and paraesthesias in the maxillary and mandibular trigeminal divisions 2 years ago. Now, he has a severe sensory loss in the intraoral and perioral territories; all the trigeminal reflexes from the maxillary and mandibular divisions are absent bilaterally, and the early and late blink reflexes are markedly abnormal bilaterally. Laser pulses directed to the perioral territory, whether set for eliciting Aδ- or C-related LEPs, failed to elicit pinprick sensations; the patient felt only burning sensations and the scalp signals had a latency compatible with unmyelinated afferent activation (N 330 ms, P 416 ms, 7 μV; N 338 ms, P 432 ms, 5 μV).

Patient 4 underwent the laser study 2 years after the onset of his Wallenberg syndrome. He still had a slight anisocoria, a slight thermal-pain hypesthesia on the left face and right limbs, and mild allodynic pain to light touch or stroking the skin of the left eyebrow and cheek. On the affected side, both pinprick and warmth thresholds to laser stimuli were increased, and both Aδ-related and C-related LEPs were absent (Fig. 4). Patient 5 had an acute Wallenberg syndrome, with thermal-pain hypesthesia on her right face and left limbs, right Bernard-Horner syndrome, dysphagia and mild cerebellar signs. As for Patient 4, on the affected side the laser thresholds were increased and both Aδ-related and C-related LEPs were absent.

## *Brain generators*

In the grand-average from the 10 subjects who underwent the multi-electrode recordings of the C-related LEPs, scalp topography was best explained by a five-dipole model (Fig. 5). Two first dipoles were localized bilaterally in the parietal opercular region, commonly identified as the secondary somatosensory area (SII). The latency of their peak activity (~200 ms) corresponded to an early negative wave disclosed by the grand-average in the temporal region (N1). A generator in the posterior portion of the anterior cingulate

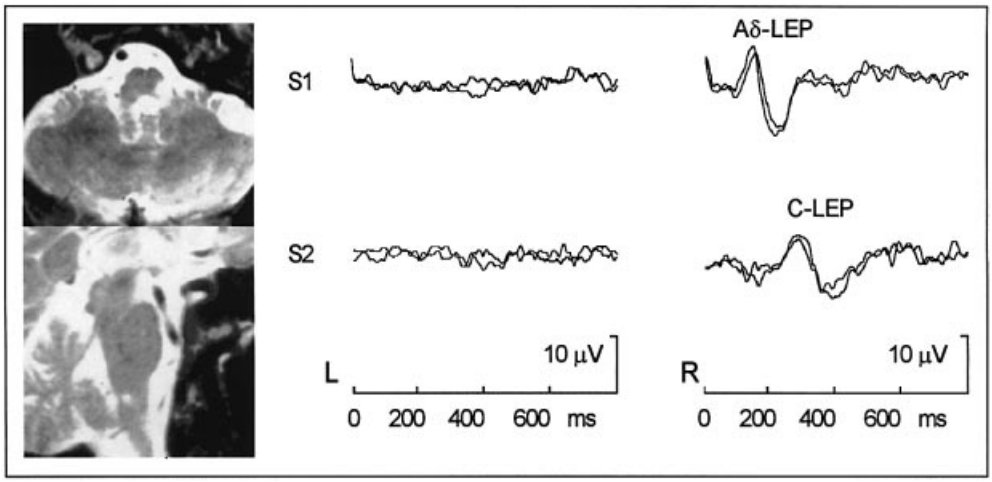


**Fig. 4** MRI and neurophysiological findings in a patient with Wallenberg syndrome (Patient 4). $T_2$-weighted MRI scans showed a small infarction in the left dorsal-lateral medulla. Laser stimuli, whether set for Aδ-fibre (76 mJ/mm$^2$, 4.9 mm$^2$) or C-fibre activation (25 mJ/mm$^2$, 177 mm$^2$) when directed to the ipsilateral face failed to evoke LEPs, whereas those directed to the contralateral face evoked normal Aδ-related and C-related LEPs. Stimulation of left (L) and right (R) sides.

cortex was activated at the latency of the main negative-positive complex (N2–P2, 280–380 ms), with the P2 wave also supported by bilateral generators in the insular region. Although the SII and insular dipoles were closely located, their time courses clearly differed (Fig. 5E). This five-dipole model yielded a 3.7% residual variance. Other possible dipolar locations, including the primary somatosensory area (SI), were tested and all yielded a far higher residual variance.

When we applied the grand-average model to traces obtained from individual subjects, allowing the dipoles to move, neither the dipolar waveforms nor the source locations differed markedly from those yielded by the grand-average model (Table 2). The inter-individual variability of source locations was similar to that found for trigeminal Aδ-related LEPs (Bromm and Chen, 1995; Kazarians *et al*., 1995).

## Discussion

Laser pulses with adequate characteristics, directed to the facial skin, selectively evoked pinprick or warmth sensations and brain potentials in latency ranges compatible with Aδ- and C-fibre conduction. The estimated conduction velocity of the afferents for C-related brain potentials was ~1.2 m/s, i.e. in the range of unmyelinated fibre conduction. Exemplary cases, serving as experimenta naturae, helped us to demonstrate that these scalp potentials are of unequivocal neural origin, are mediated by unmyelinated afferents, and follow the thermal-pain pathway in the brainstem. The most probable generators lie in the anterior cingulate gyrus and the bilateral opercular-insular regions.

### *Selectivity of stimulation*

Ample evidence shows that $CO_2$ lasers exclusively excite the most superficial free nerve endings that mediate thermal-pain sensations. Solid-state lasers, such as the Nd:YAP laser used in this study or the more widely used Nd:YAG or Tm:YAG lasers (Spiegel *et al*., 2000; Lefaucheur *et al*., 2001), having a shorter wavelength, inevitably penetrate deeper through the skin. These lasers also yield LEPs with a slightly shorter latency. Hence, the real 'selectivity' of solid-state lasers remains debatable.

A series of findings enable us to be certain that our Nd:YAP laser stimulation selectively excited Aδ or C thermal-pain receptors and that we recorded genuine neural signals related to this selective input. First, evoked sensation and LEP latency after Nd:YAP laser pulses set for Aδ-fibre activation matched those yielded by $CO_2$-lasers in previous studies (Cruccu *et al*., 1999, 2001). Secondly, with low-intensity and large-spot $CO_2$-laser pulses directed to the upper lip close to the sulcus nasus-genius (an area which in lower mammals is particularly rich in warmth receptors) (Dickenson *et al*., 1979), we evoked warmth sensations and clear LEPs at a latency similar to that yielded by the Nd:YAP (Table 1). The drawback of the $CO_2$-laser was that pulses directed to skin spots outside the upper lip inevitably co-activated Aδ-fibres, as the appearance of the Aδ-related N-160 component in the brain signals confirmed. Thirdly, with Nd:YAP laser pulses set for C-fibre activation all subjects invariably reported a purely warmth sensation. Fourthly, source analysis of the brain signal and our findings in the patients excluded a possible contamination by reflex activity (Patient 1) and confirmed that the afferent input was conveyed by unmyelinated afferents (Patients 2 and 3), and that it was mediated by the trigeminal thermal-pain nucleus in the medulla (Patients 4 and 5).

Although the stimuli we used certainly selectively activated unmyelinated afferents, we cannot exclude the possibility that nociceptive mechano-heat receptors innervated by unmyelinated fibres (CMH units) contributed to the input. We

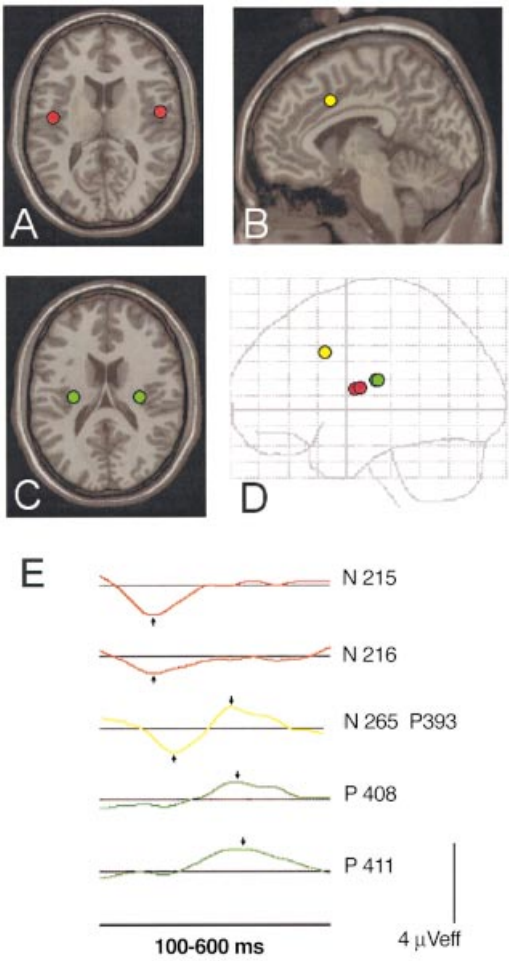


**Fig. 5** Generators of the C-related grand-average LEPs as localized by source analysis displayed on the MRI structural template. (**A**) Axial section ($z$ = 12 mm) showing the generators of the early negativity (N1), localized in the opercular parietal region bilaterally (red spots). (**B**) Sagittal section ($x$ = –5 mm) showing the generator of the main N2–P2 complex in the posterior part of the anterior cingulate gyrus (yellow spot). (**C**) Axial section ($z$ = 16 mm) showing the two additional generators of the P2 wave, localized in the insular regions (green spots). (**D**) Glass brain showing all dipoles in lateral view. (**E**) Source waveforms of the left and right opercular dipoles (red lines), anterior cingulate dipole (yellow line), and left and right insular dipoles (green lines); arrows indicate the activity peaks; latencies are shown near each waveform.

believe that most of the afferent volley arose from warmth rather than CMH receptors because of the low rise in skin temperature induced by the laser pulse (always lower than 40°C) and because our subjects explicitly reported a non-burning warmth sensation. The brain signals provide no help in this case, because thermal and nociceptive sensations seem to have similar brain generators. Indeed, in Patient 2—who, as expected in trigeminal neuropathy (Lecky *et al*., 1987) lacked myelinated fibres—we could increase the stimulus intensity without eliciting any Aδ-related potential: the patient felt clear burning sensations and the brain signals did not differ from those seen with the stimulus characteristics optimal for eliciting warmth sensations (Fig. 3). Similarly, in the other patient with trigeminal neuropathy (Patient 3), we could increase the stimulus intensity without eliciting any Aδ-related potential, but this patient felt only burning sensations regardless of stimulus characteristics.

### *Unmyelinated trigeminal afferents*

The measurement of conduction velocity must be considered an estimate, because, instead of always stimulating the same spot and recording the afferent volley at different sites along the nerve (which cannot be done even with microneurography), we stimulated different spots and measured the latency of the brain potentials. By taking care to provide a quantitatively similar input, thus minimizing differences in spatial summation at central synapses, we obtained LEPs of similar amplitude. Calculating the slope of the regression line evaluates statistically the reliability of the estimate of velocity and, by minimizing the weight of individual measures, increases their statistical power (Cruccu *et al*., 2000; Iannetti *et al*., 2003).

The conduction velocity that we found, 1.2 m/s, clearly comes within the 0.4–2.5 m/s range of unmyelinated fibres. In Patient 2, the unmyelinated fibres of the supraorbital nerve ranged from 0.2 to 1.4 μm in diameter, with one peak between 0.4 and 0.8 μm, consistent with our estimate of conduction velocity in the supraorbital nerve. Calculated using Gasser's ratio (1.7), the expected velocity for that diameter peak would be 0.7–1.4 m/s (Gasser, 1950, 1955).

The available information on unmyelinated-fibre conduction in the trigeminal nerve comes from two studies only: one using electrical stimulation and microneurographic recordings in man (0.6–1.4 m/s; Nordin, 1990) and the other investigating polymodal nociceptors in the monkey (0.8 m/s; Beitel and Dubner, 1976).

The results of studies in the human limb nerves suggest that velocities of thermal and nociceptive C fibres overlap. Studies using C-related LEPs after laser stimulation of the upper arm reported a similar mean velocity of 1.2 m/s and 1.3 m/s for the nociceptive (Opsommer *et al*., 1999; Tran *et al*., 2002) and 1.3 m/s for the thermal fibres (Towell *et al*., 1996). In a human study still using C-related LEPs, Magerl *et al*. (1999) estimated a velocity of 2.4 m/s, but these investigators left open the question whether the afferents were thermal or nociceptive. Studies using warm stimuli (contact thermode) and measuring reaction times estimated a velocity of 0.5–1.5 m/s (Yarnitsky and Ochoa, 1991; Opsommer *et al*., 1999). Microneurographic data on human thermal afferents are based on very few fibres, probably because of the small number of this kind of afferents. In a large number of mechano-insensitive (CH) and mechano-thermal (CMH) C-fibre nociceptors, the mean velocities were 0.8 and 1.0 m/s (Weidner *et al*., 1999).

In studies that compared thermal and nociceptive afferents in monkeys, in contrast, the conduction velocity was

**Table 2** *Talairach coordinates of C-LEP sources (mean ± SD)*

| | | Dipole 1 | | | Dipole 2 | | | Dipole 3 | | | Dipole 4 | | | Dipole 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | RV | *x* | *y* | *z* | *x* | *y* | *z* | *x* | *y* | *Z* | *x* | *y* | *z* | *x* | *y* | *z* |
| Grand-average | 3.7 | –47 | 6 | 11 | 54 | 9 | 12 | –6 | 27 | 36 | –34 | –2 | 18 | 36 | –1 | 18 |
| Individual subjects | 7.96 | –46.7 | 6.4 | 9.8 | 52.3 | 7.2 | 11.1 | –2.7 | 24.2 | 31.7 | –33.1 | –7.1 | 16.4 | 35.5 | –3.3 | 18.1 |
| SD | 2.53 | 0.5 | 6.9 | 8.3 | 2.2 | 4.1 | 1.3 | 6.4 | 12 | 8.1 | 1.8 | 5.3 | 3.2 | 2.2 | 4.4 | 0.9 |

RV = residual variance.

consistently higher in thermal than in nociceptive unmyelinated afferents (1.1–1.2 m/s versus 0.8 m/s) (Darian-Smith *et al*., 1979; LaMotte and Campbell, 1978).

Although these data suggest that the conduction velocity found in this study fits more with thermal than with nociceptive afferents, we must consider that the bulk of the available literature refers only to limb nerves. The conduction velocity of the trigeminal afferents is also only one of their peculiar physiological properties (Cruccu *et al*., 1987; Leandri *et al*., 1998).

## *Pathways*

The time spent for receptor activation with laser stimulation (~40 ms) has been measured with microneurographic recordings from the human superficial radial nerve (Bromm and Treede, 1991). The activation times for myelinated and unmyelinated receptors after laser stimulation of the facial skin are unknown. The times for receptor activation may be longer on the hand than on the facial skin, which is thinner and probably has a higher receptor density (Whitton and Everall, 1973; Agostino *et al*., 2000).

The small thermal-pain afferents project to the trigeminal nucleus caudalis. From measurements on adult skull and stereotactic atlas, the route from the supraorbital region to the caudalmost trigeminal complex at C1 level (the maximum possible distance along the primary sensory neuron) amounts to 140 mm (Schaltenbrand and Wahren, 1977; Paxinos *et al*., 1995; Leandri *et al*., 1998). In our experiment on conduction velocity, we estimated a velocity of 1.2 m/s for the unmyelinated trigeminal afferents (Fig. 1). This velocity would yield a conduction time of 117 ms along the primary neuron. The distance from C1 to the thalamus is ~65 mm; the conduction velocity is usually higher in the central tracts than in the periphery: that for the laser-elicited C input along the spinal cord has been estimated as 2.2–2.9 m/s (Iannetti *et al*., 2003; Tran *et al*., 2002); at this velocity 22–30 ms should be spent along the secondary neuron. No more than 180–187 ms (receptor 40 ms, primary neuron 117 ms, secondary neuron 22–30 ms) should elapse from the stimulus onset to the arrival of afferent impulses at the thalamus. The peak latency of the main negative component of C-related LEPs was 289 ms, which leaves some 100–110 ms for conduction and processing within the brain, a delay certainly unjustified by thalamocortical conduction time alone.

Given the reported estimates of afferent conduction velocity along the primary neuron (9–15 m/s, Kenton *et al*., 1980; Bromm and Treede, 1991) and secondary neuron (10–20 m/s, Kakigi and Shibasaki, 1991; Cruccu *et al*., 2000) for the laser-elicited Aδ input, the Aδ impulses would take 22 ms with the lowest velocities and 12 ms with the highest on the same route as that calculated for the C input. Considering the time spent for receptor activation (40 ms), 52–62 ms elapse before the impulses reach the thalamus. The peak latency of the main component of the Aδ-related LEPs (corresponding to the so-called N2 after hand stimulation) was 166 ms. Hence the intracerebral times of the Aδ-related LEPs (166 minus 52–62 ms = 102–112 ms) were similar to those of the C-related LEPs.

According to the source analysis (Fig. 5), the generator of the main components (N2–P2) of our C-related LEPs was located in the posterior part of the anterior cingulate cortex; the P2 wave also being supported by bilateral generators in the insula, i.e. in the same regions that generate the N2–P2 components of the Aδ-related LEPs after hand, foot, and face stimulation (Tarkka and Treede, 1993; Bromm and Chen, 1995; Valeriani *et al*., 1996, 2000). The generators of the early waves (N1–P1) of the Aδ-related LEPs after hand or face stimulations have been located in lateral areas—mostly the secondary somatosensory cortex (SII) (Tarkka and Treede, 1993; Bromm and Chen, 1995; Valeriani *et al*., 1996, 2000). The long intracerebral times that we estimated (>100 ms) suggest that the C afferent input, before reaching the cingulate cortex, is conveyed and processed (like the Aδ input) elsewhere. Although we did not identify an early activity preceding the N2 wave in the standard individual recordings, the grand-average did reveal a negative wave at ~200 ms in the temporal leads and source analysis yielded the highest probabilities for a generator in the SII region (Fig. 5).

Consistently, in recent studies on functional MRI (fMRI) activation by laser pulses applied to the hand (Bornhovd *et al*., 2002; Buchel *et al*., 2002), the anterior cingulate cortex (and the dorsolateral prefrontal cortex) discriminated between warm and non-perceived stimuli; however, the SII-insular regions also seemed to show activation by warm stimuli. Although magnetoencephalography (MEG) succeeds in detecting field sources in SI generated by Aδ painful inputs (Ploner *et al*., 1999, 2002; Kanda *et al*., 2000; Inui *et al*., 2002; Tran *et al*., 2002), the MEG results about a possible field source generated by C-fibre input in SI are still

controversial (Ploner *et al*., 2002; Tran *et al*., 2002). Most investigators agree that the main generators of the LEPs after C-nociceptor stimulation are the same brain regions (anterior cingulate cortex and SII-insular areas) that we activated with warm stimuli (Opsommer *et al*., 2001; Ploner *et al*., 2002).

Involvement of the same brain regions in processing warm and noxious laser stimuli lends support to the view that heat–pain perception requires integrated information from both nociceptive and thermal channels (Defrin *et al*., 2002).

## *Conclusion and clinical significance*

The distinctive feature of this study is that it provides objective evidence of brain signals certainly related to the activation of unmyelinated trigeminal afferents. It also describes their physiological properties, including afferent conduction velocity and brain generators.

Because the short conduction distance minimizes the problem of signal dispersion along slow-conducting unmyelinated afferents and probably because the area has a high receptor density, the trigeminal territory is advantageous for studying C-related LEPs. Few trials are sufficient to obtain reproducible brain signals and no damage at all is caused to the skin. Under fibreoptic guidance, the laser beam can be directed to any facial region. Although the brain signals that can be measured easily in patients (i.e. the main N2/P2 complex), rather than representing the arrival of the afferent input at the sensory-discriminative cortex probably reflect the activity of non-sensory-modality-specific processing in the cingulate cortex, they nonetheless provide a reliable assessment of the unmyelinated fibre pathways. As we verified in our patients, these responses are sensitive to peripheral and central lesions of the trigeminal thermal-pain pathways. Our technique may prove clinically useful in any condition affecting these pathways.

### References

Agostino R, Cruccu G, Iannetti GD, Romaniello A, Truini A, Manfredi M. Topographical distribution of pinprick and warmth thresholds to CO2 laser stimulation on the human skin. Neurosci Lett 2000; 285: 115–8.

Arendt-Nielsen L. Second pain event related potentials to argon laser stimuli: recording and quantification. J Neurol Neurosurg Psychiatry 1990; 53: 405–10.

Beitel RE, Dubner R. Response of unmyelinated (C) polymodal nociceptors to thermal stimuli applied to monkey's face. J Neurophysiol 1976; 39: 1160–75.

Bornhovd K, Quante M, Glauche V, Bromm B, Weiller C, Buchel C. Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study. Brain 2002; 125: 1326–36.

Bragard D, Chen AC, Plaghki L. Direct isolation of ultra-late (C-fibre) evoked brain potentials by CO2 laser stimulation of tiny cutaneous surface areas in man. Neurosci Lett 1996; 209: 81–4.

Bromm B, Chen AC. Brain electrical source analysis of laser evoked potentials in response to painful trigeminal nerve stimulation. Electroencephalogr Clin Neurophysiol 1995; 95: 14–26.

Bromm B, Treede RD. Laser-evoked cerebral potentials in the assessment of cutaneous pain sensitivity in normal subjects and patients. Rev Neurol (Paris) 1991; 147: 625–43.

Bromm B, Neitzel H, Tecklenburg A, Treede RD. Evoked cerebral potential correlates of C-fibre activity in man. Neurosci Lett 1983; 43: 109–14.

Buchel C, Bornhovd K, Quante M, Glauche V, Bromm B, Weiller C. Dissociable neural responses related to pain intensity, stimulus intensity, and stimulus awareness within the anterior cingulate cortex: a parametric single-trial laser functional magnetic resonance imaging study. J Neurosci 2002; 22: 970–6.

Cruccu G, Inghilleri M, Manfredi M, Meglio M. Intracranial stimulation of the trigeminal nerve in man. III. Sensory potentials. J Neurol Neurosurg Psychiatry 1987; 50: 1323–30.

Cruccu G, Berardelli A, Inghilleri M, Manfredi M. Functional organization of the trigeminal motor system in man. A neurophysiological study. Brain 1989; 112: 1333–50.

Cruccu G, Romaniello A, Amantini A, Lombardi M, Innocenti P, Manfredi M. Assessment of trigeminal small-fiber function: brain and reflex responses evoked by CO2-laser stimulation. Muscle Nerve 1999; 22: 508–16.

Cruccu G, Iannetti GD, Agostino R, Romaniello A, Truini A, Manfredi M. Conduction velocity of the human spinothalamic tract as assessed by laser-evoked potentials. Neuroreport 2000; 11: 3029–32.

Cruccu G, Leandri M, Iannetti GD, Mascia A, Romaniello A, Truini A, et al. Small-fiber dysfunction in trigeminal neuralgia. Carbamazepine effect on laser evoked potentials. Neurology 2001; 56: 1722–6.

Darian-Smith I, Johnson KO, LaMotte C, Shigenaga Y, Kenins P, Champness P. Warm fibers innervating palmar and digital skin of the monkey: responses to thermal stimuli. J Neurophysiol 1979; 42: 1297–315.

Defrin R, Ohry A, Blumen N, Urca G. Sensory determinants of thermal pain. Brain 2002; 125: 501–10.

Dickenson AH, Hellon RF, Taylor DC. Facial thermal input to the trigeminal spinal nucleus of rabbits and rats. J Comp Neurol 1979; 185: 203–9.

Evans AC, Collins DL, Mills SR, Brown ED, Kelly RL, Peters TM. 3D statistical neuroanatomical models from 305 MRI volumes. In: Proceedings of the IEEE Nuclear Science Symposium and Medical Imaging. 1993. p. 1813–17.

Gasser HS. Unmedullated fibers originating in dorsal root ganglia. J Gen Physiol 1950; 33: 651–90.

Gasser HS. Properties of dorsal root unmedullated fibers on the two sides of the ganglion. J Gen Physiol 1955; 38: 709–28.

Granot M, Buskila D, Granovsky Y, Sprecher E, Neumann L,

Yarnitsky D. Simultaneous recording of late and ultra-late pain evoked potentials in fibromyalgia. Clin Neurophysiol 2001; 112: 1881–7.

Green BG, Cruz A. Warmth-insensitive fields: evidence of sparse and irregular innervation of human skin by the warmth sense. Somatosens Mot Res 1998; 15: 269–75.

Iannetti GD, Truini A, Romaniello A, Galeotti F, Rizzo C, Manfredi M, et al. Evidence for a specific spinal pathway for the sense of warmth in humans. J Neurophysiol 2003; 89: 562–70.

Inui K, Tran TD, Qiu Y, Wang X, Hoshiyama M, Kakigi R. Pain-related magnetic fields evoked by intra-epidermal electrical stimulation in humans. Clin Neurophysiol. 2002; 113: 298–304.

Kakigi R, Shibasaki H. Estimation of conduction velocity of the spinothalamic tract in man. Electroencephalogr Clin Neurophysiol 1991; 80: 39–45.

Kakigi R, Shibasaki H, Kuroda Y, Neshige R, Endo C, Tabuchi K, et al. Pain-related somatosensory evoked potentials in syringomyelia. Brain 1991; 114: 1871–89.

Kakigi R, Shibasaki H, Ikeda T, Neshige R, Endo C, Kuroda Y. Pain-related somatosensory evoked potentials following CO2 laser stimulation in peripheral neuropathies. Acta Neurol Scand 1992; 85: 347–52.

Kazarians H, Scharein E, Bromm B. Laser evoked brain potentials in response to painful trigeminal nerve activation. Int J Neurosci 1995; 81: 111–22.

Kanda M, Nagamine T, Ikeda A, Ohara S, Kunieda T, Fujiwara N, et al. Primary somatosensory cortex is actively involved in pain processing in human. Brain Res 2000; 853: 282–9.

Kenton B, Coger R, Crue B, Pinsky J, Friedman Y, Carmon A. Peripheral fiber correlates to noxious thermal stimulation in humans. Neurosci Lett 1980; 17: 301–6.

LaMotte RH, Campbell JN. Comparison of responses of warm and nociceptive C-fiber afferents in monkey with human judgments of thermal pain. J Neurophysiol 1978; 41: 509–28.

Lankers J, Frieling A, Kunze K, Bromm B. Ultralate cerebral potentials in a patient with hereditary motor and sensory neuropathy type I indicate preserved C-fibre function. J Neurol Neurosurg Psychiatry 1991; 54: 650–2.

Leandri M. Generator sites of early scalp potentials evoked from the three trigeminal branches. J Neurosurg 1998; 88: 718–25.

Lecky BR, Hughes RA, Murray NM. Trigeminal sensory neuropathy. A study of 22 cases. Brain 1987; 110: 1463–85.

Lefaucheur JP, Debray S, Jarry G. Laser evoked potentials using the Nd: YAG laser. Muscle Nerve 2001; 24: 496–501.

Magerl W, Ali Z, Ellrich J, Meyer RA, Treede RD. C- and Aδ-fibre components of heat-evoked cerebral potentials in healthy human subjects. Pain 1999; 82: 127–37.

Nordin M. Low-threshold mechanoreceptive and nociceptive units with unmyelinated (C) fibres in the human supraorbital nerve. J Physiol 1990; 426: 229–40.

Ongerboer de Visser BW, Cruccu G. Neurophysiologic examination of the trigeminal, facial, hypoglossal, and spinal accessory nerves in cranial neuropathies and brain stem disorders. In: Brown WF, Bolton CF, editors. Clinical electromyography. 2nd ed. Boston: Butterworth-Heinemann; 1993. p. 61–92.

Opsommer E, Masquelier E, Plaghki L. Determination of nerve conduction velocity of C-fibres in humans from thermal thresholds to contact heat (thermode) and from evoked brain potentials to radiant heat ($CO_2$ laser). Neurophysiol Clin 1999; 29: 411–22.

Opsommer E, Weiss T, Plaghki L, Miltner WH. Dipole analysis of ultralate (C-fibres) evoked potentials after laser stimulation of tiny cutaneous surface areas in humans. Neurosci Lett 2001; 298: 41–4.

Paxinos G, Huang X-F. Atlas of the human brainstem. San Diego: Academic Press; 1995.

Pennisi E, Cruccu G, Mostarda M, Piccotti A, Palladini G, Manfredi M. Supraorbital nerve: histological and neurophysiological correlations with pain in three patients with trigeminal neuropathy and a control subject. Ital J Neurol Sci 1997; 18: 233.

Ploner M, Schmitz F, Freund HJ, Schnitzler A. Parallel activation of primary and secondary somatosensory cortices in human pain processing. J Neurophysiol 1999; 81: 3100–4.

Ploner M, Gross J, Timmermann L, Schnitzler A. Cortical representation of first and second pain sensation in humans. Proc Natl Acad Sci USA 2002; 99: 12444–8.

Qiu Y, Inui K, Wang X, Tran TD, Kakigi R. Conduction velocity of the spinothalamic tract in humans as assessed by CO(2) laser stimulation of C-fibers. Neurosci Lett 2001; 311: 181–4.

Romaniello A, Valls-Solé J, Iannetti GD, Truini A, Manfredi M, Cruccu G. Nociceptive quality of the laser-evoked blink reflex in humans. J Neurophysiol, 2002; 87: 1386–94.

Schaltenbrand G, Wahren W. Atlas for stereotaxy of the human brain. 2nd ed. Stuttgart: Thieme; 1977.

Scherg M. Fundamentals of dipole source potential analysis. In: Grandoni F, Hoke M, Romani GL, editors. Auditory evoked magnetic fields and electric potentials. Advances in audiology, Vol. 6. Basel: Karger; 1990. p. 40–69.

Spiegel J, Hansen C, Treede RD. Clinical evaluation criteria for the assessment of impaired pain sensitivity by thulium-laser evoked potentials. Clin Neurophysiol 2000; 111: 725–35.

Tarkka IM, Treede RD. Equivalent electrical source analysis of pain-related somatosensory evoked potentials elicited by a CO2 laser. J Clin Neurophysiol 1993; 10: 513–9.

Tillman DB, Treede RD, Meyer RA, Campbell JN. Response of C fibre nociceptors in the anaesthetized monkey to heat stimuli: estimates of receptor depth and threshold. J Physiol 1995; 485: 753–65.

Towell AD, Purves AM, Boyd SG. CO2 laser activation of nociceptive and non-nociceptive thermal afferents from hairy and glabrous skin. Pain 1996; 66: 79–86.

Tran TD, Inui K, Hoshiyama M, Lam K, Qiu Y, Kakigi R. Cerebral activation by the signals ascending through unmyelinated C-fibers in humans: a magnetoencephalographic study. Neuroscience 2002; 113: 375–86.

Treede RD, Meier W, Kunze K, Bromm B. Ultralate cerebral

potentials as correlates of delayed pain perception: observation in a case of neurosyphilis. J Neurol Neurosurg Psychiatry 1988; 51: 1330–3.

Treede RD, Lankers J, Frieling A, Zangemeister WH, Kunze K, Bromm B. Cerebral potentials evoked by painful, laser stimuli in patients with syringomyelia. Brain 1991; 114: 1595–607.

Treede RD, Meyer RA, Raja SN, Campbell JN. Evidence for two different heat transduction mechanisms in nociceptive primary afferents innervating monkey skin. J Physiol 1995; 483: 747–58.

Valeriani M, Rambaud L, Mauguière F. Scalp topography and dipolar source modelling of potentials evoked by CO2 laser stimulation of the hand. Electroencephalogr Clin Neurophysiol 1996; 100: 343–53.

Valeriani M, Restuccia D, Barba C, Le Pera D, Tonali P, Mauguière F. Sources of cortical responses to painful CO2 laser skin stimulation of the hand and foot in the human brain. Clin Neurophysiol 2000; 111: 1103–12.

Valeriani M, Le Pera D, Tonali P. Characterizing somatosensory evoked potential sources with dipole models: advantages and limitations. Muscle Nerve 2001; 24: 325–39.

Weidner C, Schmelz M, Schmidt R, Hansson B, Handwerker HO, Torebjork HE. Functional attributes discriminating mechano-insensitive and mechano-responsive C nociceptors in human skin. J Neurosci 1999; 19: 10184–90.

Whitton JT, Everall JD. The thickness of the epidermis. Br J Dermatol 1973; 89: 467–76.

Yarnitsky D, Ochoa JL. Warm and cold specific somatosensory systems. Psychophysical thresholds, reaction times and peripheral conduction velocities. Brain 1991; 114: 1819–26.

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